Comparative Performance of Sand and Longleaf Pines on a *Phytophthora Cinnamomi*-Infested Sandhill in West Florida

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ABSTRACT. *Survival, but not growth, of Choctawhatchee sand pine exceeded that of Ocala sand pine on a* Phytophthora cinnamomi*-infested sandhill in west Florida. However, both varieties exhibited relatively high levels of susceptibility to root disease and mortality caused by P. cinnamomi. Longleaf pine survived significantly better than either variety of sand pine and exhibited an apparent resistance to infection by* P. cinnamomi. *South. J. Appl. For. 17(2): 90–95.*

Sand pine (*Pinus clausa* [Chapm.] Vasey) is a relatively small, short-lived, southern yellow pine native to Florida's sandhills. Botanists and foresters have generally recognized two distinct varieties of sand pine: Choctawhatchee (*P. clausa* var. *immuginata* Ward), native to Florida's western panhandle, and Ocala (*P. clausa* var. *clausa*), native to peninsular Florida. Interest in commercial management of the species is driven largely by its ability to grow well on deep, droughty, infertile, acid sands (Balmer 1973, Brendemuehl 1981, Burns 1972, Hebb 1982, Outcalt and Brendemuehl 1985, Preston and Price 1979) and its potential as a Christmas tree (Beckwith and Pritchett 1968).

Root diseases, caused by a variety of fungi acting alone or in combination, frequently impede the successful management of sand pine (Barnard 1988, Barnard et al. 1985a,b, Ross 1973, Ross and Marx 1972). Perhaps the most serious of these diseases is Phytophthora root rot caused by *Phytophthora cinnamomi* Rands (Barnard 1988, Barnard et al. 1985 a, b, Ross 1973, Ross and Marx 1972), the same fungus implicated in the littleleaf disease syndrome of shortleaf (*P. echinata* Mill.) and loblolly (*P. taeda* L.) pines (Mistretta 1984). Ross and Marx (1972) demonstrated the pathogenicity of *P. cinnamomi* on sand pine, and Barnard et al. (1985a) found this organism to be the most frequently detected root pathogenic fungus in sand pine plantations with root disease. Evidence suggests that Phytophthora root rot in sand pine plantations sometimes results from carrying infected planting stock from the nursery to the field (Barnard 1988, Barnard et al. 1985a,b). Fine-textured, poorly drained soils or soils with shallow, impervious layers, or evidence of poor internal drainage and/or aeration (mottling, etc.) are generally regarded as favored habitat for the pathogenic activity of *P. cinnamomi* (Zentmyer 1980). Not surprisingly, such sites are particularly conducive to root rot of sand pine (Barnard et al. 1985a, Ross 1973, Ross and Marx 1972).

In 1984, while evaluating potential sand pine seed orchard sites with Champion International Corp., we confirmed the presence of *P. cinnamomi* in the soil beneath a stagnant 22-yr-old slash pine (*P. elliottii* Engelm.) plantation in western Florida. The soil was classified on existing Soil Conservation Service maps as a deep Lakeland sand and was therefore presumably a silviculturally acceptable sand pine site (Brendemuehl 1981, Hebb 1982, Outcalt and Brendemuehl 1985, Preston and Price 1979). However, seed orchard plans for this site were abandoned because of concern over potential losses to *P. cinnamomi*. Instead, we established a test planting of sand and longleaf (*P. palustris* Mill.) pines. Our primary objective was to evaluate the performance of both Choctawhatchee (CSP) and Ocala (OSP) sand pines, under challenge by *P. cinnamomi* on a good sand pine site, thereby removing poor soil drainage and lack of root aeration as primary causes of any developing root disease. Longleaf pine was included for comparison because of its comparable suitability for sandhill planting (Shoulders 1990)

and its generally recognized resistance to diseases (Hodges 1974, Kuhlman 1970, Powers 1975, Snow et al. 1990).

Materials and Methods

Site Characterization

The presence of *P. cinnamomi* in the study site soil was verified on each of four dates up to and including the time of plantation establishment (Tables 1 and 2). Composited soil cores (ca. 25 cm deep × 2.0 cm diameter) were collected, sealed in plastic bags, and transported on ice to the laboratory. Soil core extractors were sterilized between each sample on the first three sampling dates by rinsing and flaming with 95% ethanol. On the fourth sampling date, single soil cores from each sampling locus (i.e., seedling rhizosphere) were extracted and discarded prior to extraction of composite sample cores to minimize carryover of *P. cinnamomi* from the previous sampling locus. In the laboratory, composite soil samples were thoroughly mixed, and sixteen 0.5 cc (approx.) subsamples of each were carefully placed into 1.0 cm diameter "wells" (Figure 1) cut with a cork borer in plates of a hymexazol-amended (Masago et al. 1977, Tsao and Guy 1977) *Phytophthora*-selective medium (Kannwischer and Mitchell 1981). Two to three drops of sterile deionized water were added to each soil-filled well, and plates were incubated in the dark at room temperature for 3 days prior to examination for colonies of *P. cinnamomi*.

Soil nutritional and textural characteristics were assessed according to standard laboratory procedures using soil samples collected at the time of planting. Residuals of the 10 seedling-rhizosphere samples from each of the 20 sand pine blocks (Tables 1 and 2) were composited by block and processed by the University of Florida's Forest Soils Laboratory.

Site Preparation

Preparation of the study site consisted of a whole-tree harvest (trees chipped on site) of the existing slash pine plantation in July followed by a rotary mowing of oak (primarily *Quercus laevis* Walt.) sprouts in November 1986.

Seedling Production and Quality Assessments

All seedlings used in this study were operationally grown in the Lee Nursery (Champion International Corp.; currently Superior Trees, Inc.) at Lee, FL. Half of the seedlings, both sand and longleaf pines, were inoculated with vegetative ectomycorrhizal inoculum of *Pisolithus tinctorius* Pers. [Pt] (Marx et al. 1984) at a rate of 1.0 l of inoculum per 40 ft^2 of nursery seedbed. Effectiveness of the Pt inoculations was evaluated by visual assessment of ectomycorrhizae (Marx et al. 1984) at mid-season (August 29, 1986) and at lifting (January 5, 1987) just prior to plantation establishment. Seedling heights (sand pine only) and root collar diameters (both species) were determined at lifting for each of two randomly selected sets of 10 seedlings from each species and Pt-inoculation treatment. In addition, root pieces from five randomly selected seedlings from each treatment were plated onto the *Phytophthora*-selective medium to determine the presence or absence of *P. cinnamomi*.

Plantation Establishment and Evaluations

On the day seedlings were lifted, one-half of the seedlings of each species and Pt-inoculation treatment were root-dipped in a kaolin clay slurry; the other half were dipped in a benomyl-amended (5% ai) kaolin clay slurry (Kais et al. 1986). Seedlings were refrigerated overnight in kraft paper (KP) bags and hand-planted with dibbles the following day in 49-tree rectangular plots (7 × 7 trees @ 6 × 10 ft spacing). Five replicate plots were established for each treatment in a randomized complete block design.

The study site was visited at irregular intervals over the next 3 yr. On each visit, all dead/dying trees were carefully excavated, and their root systems were transported on ice to the laboratory for evaluation. In the laboratory, root systems were examined for resinosis typical of that induced by *P. cinnamomi* infections (Barnard 1988), and portions of each root system were plated on the *Phytophthora*-selective medium. Plates were examined for *P. cinnamomi* after 3–5 days of dark incubation at room temperature. Resin-soaked roots of several seedlings were also plated on acidified (3.3 ml of 50% lactic acid/L) potato-dextrose agar and Nash and Snyder's (1962) *Fusarium*-selective medium to check for possible pitch canker and/or other *Fusarium* infections which are also capable of inducing root resinosis in pines.

The heights and basal stem diameters of all surviving trees were measured at the end of the 3-yr study period.

Results and Discussion

Typical of unproductive sandhills (Brendemuehl 1973, Burns and Hebb 1972), rhizosphere soils on our study site contained relatively low levels of extractable nutrients. Nutrient concentrations in parts per million averaged 286 N, 0.95 P, 7.1 K, 17.6 Ca, 4.3 Mg, 17.3 Mn, 191 Al, 24.9 Fe, 0.19 Cu, and 0.05 Zn.

Table 1. Recovery of *Phytophthora cinnamomi* from a deep lakeland sand in west Florida.

Sampling date	No. samples	Soil cores per sample	Sample distribution	Samples positive No.	Samples positive %
4/17/84	3	10	random	2	66.6
6/19/84	25	10	systematic/line plot[1]	17	68.0
12/17/85	20	10	systematic/line plot[1]	14	70.0
1/7/87[2]	200	4	systematic/seedling rhizospheres[3]	125	62.5

[1] Evenly spaced across 5 ac study site.
[2] Study planting established 1/6/87.
[3] Samples in rhizospheres of each of 10 systematically distributed Choctawhatchee and Ocala sand pines in each of 10 plots per variety (ref. Table 2).

Table 2. Occurrence and distribution of *Phytophthora cinnamomi* in rhizospheres of planted sand pines on a west Florida sandhill (Sample date: January 7, 1987).

Variety	Treatment	No. samples	Measure[a]	Rep. no. 1	2	3	4	5	Mean
Ocala	Pt	50		10	60	80	70	70	58
			II	4.4	11.3	29.4	16.3	29.4	18.2
	NI	50		60	70	40	60	30	52
			II	35.0	20.0	13.8	28.1	11.9	21.8
Choctawhatchee	Pt	50		90	90	90	20	80	74
				28.8	32.5	36.3	6.3	30.0	26.8
	NI	50	I	60	80	90	50	50	66
			II	27.5	36.9	36.9	16.9	11.3	25.9

I = Percentage of rhizospheres from which *P. cinnamomi* was isolated out of 10 sampled per replicate.
II = Percentage of agar plate soil wells (ref. Fig. 1) positive for *P. cinnamomi* out of a total of 16 wells (2 plates) per rhizosphere sample.

Particle composition was 89% sand, 7% silt, and 4% clay, and repeated borings with a bucket auger revealed no obvious textural changes within 150 cm (length of auger handle) of the soil surface. Lakeland Soil Series, by definition, are characterized by sands in excess of 200 cm in depth. Nonetheless, the site was well colonized by *P. cinnamomi* prior to and at the time of plantation establishment (Tables 1 and 2).

At planting, Pt and other ectomycorrhizae were more numerous on inoculated than on noninoculated seedlings (Table 3). The total percentages of ectomycorrhizal feeder roots, however, were generally unimpressive and often less than those recorded the previous August, possibly because of rootlet losses sustained during lifting and handling. Seedlings of a given species or variety differed very little in size between inoculated and noninoculated treatments. All seedlings, as they left the nursery, were free of infection by *P. cinnamomi* (Table 3).

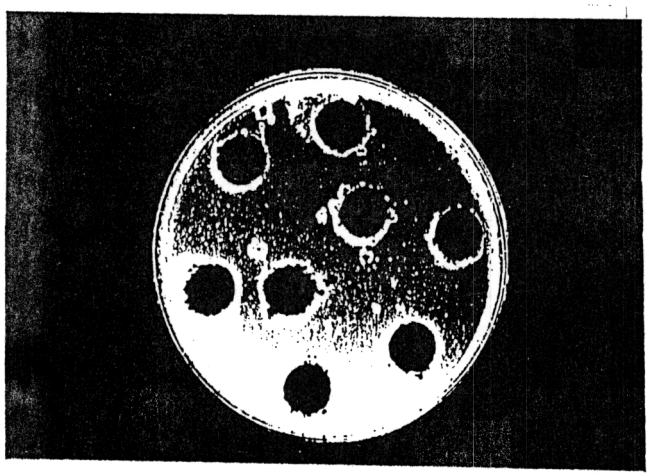

Figure 1. Soil-well plate of a *Phytophthora*-selective medium employed to detect and quantify relative levels of soilborne *Phytophthora cinnamomi* on a west Florida sandhill.

Pt-inoculation, in conjunction with the benomyl root treatment, significantly increased treatment volume index for longleaf pine. Longleaf pines treated in this manner had a treatment volume index comparable to that of Choctawhatchee sand pine at the end of the study period. Otherwise, there were no meaningful differences in field performance between Pt-inoculated and noninoculated seedlings within a pine species and/or variety (Table 4). These results, coupled with a lack of discernible differences with respect to the occurrence of root disease symptoms (i.e., root resinosis) and laboratory recovery of *P. cinnamomi*, caused us to pool data from inoculated and noninoculated seedlings within each species and/or variety for purposes of root disease assessment.

Benomyl-treated longleaf pines exhibited less evidence of infection by *Mycosphaerella dearnessii* Barr (=*Scirrhia acicola* [Dearn] Siggers), the brown spot needle blight fungus, than did nontreated checks through the first year in the field. However, first-year disease pressure was not particularly high, and differences in brown spot infection between check and benomyl-treated trees were not substantial during the second and third years in the field.

Large numbers of seedlings died due to apparent transplant shock or were killed by an outbreak of pales (*Hylobius pales* [Herbst]) and pitcheating (*Pachylobius picivorus* [Germar]) weevils during the first few months (168 days) following outplanting (Figure 2). Accordingly, early seedling mortality could not be attributed solely to infections by *P. cinnamomi*, despite the fact that some 19% of 41 dead/dying Choctawhatchee and 39% of 61 dead/dying Ocala sand pines collected through this period yielded the fungus upon isolation. Indeed, none of the dead and dying seedlings (including longleaf pines) analyzed during this period exhibited root resinosis typical of that observed on *P. cinnamomi*-infected sand pines. Consequently, root rot assessments were limited to trees that died subsequent to June 23, 1987, after all weevil activity had ceased and the impact of transplant shock had subsided.

Figure 2 displays the progression of seedling mortality over the 3-yr study period. Cumulative mortality is depicted both as a percentage of the total number of trees planted for each species and/or variety (i.e., 490 CSP, 490 OSP, and 980 longleaf) (Figure 2-A) and as a percentage of the trees living on sample

Table 3. Comparative mycorrhizal, size, and *Phytophthora cinnamomi*-infection status of sand and longleaf pine seedlings employed in field outplant study.

Treatment	Mycorrhizal Assessment				Size & disease assessment[2]		
	% Seedlings with Pt	% ectomycorrhizae			Mean Stem		
			Total	Pt index[3]	Ht(cm)	Dia(mm)	% Seedlings w/*P. cinnamomi*
Pt-Inoculated							
Longleaf	80/70	30/4	35/12	69/26		10.2	0
Choctawhatchee	100/100	30/17	35/30	86/57	25.8	3.7	0
Ocala	60/95	10/10	15/18	40/51	26.2	3.9	0
Noninoculated							
Longleaf	0/0	0/0	15/7	0/0	—	10.0	0
Choctawhatchee	0/0	0/0	10/26	0/0	22.7	3.3	0
Ocala	0/0	0/0	5/9	0/0	26.8	3.4	0

1 Assessment @ Aug. 29, 1986/assessment @ Jan. 5–6, 1987.
2 Assessment @ time of lifting/outplanting; Jan 5–6, 1987.
3 Pt index = % of seedling feeder roots with Pt/% seedling feeder roots with all ectomycorrhizae (including Pt) × % seedlings with any Pt (column 1

dates immediately preceding each successive site visit and mortality assessment (subsequent to June 23, 1987) (Figure 2-B). Pronounced species and varietal differences are evident. OSP seedlings clearly exhibited the highest level (Table 4, Figure 2-A) and rate (i.e., slope of curve; Figure 2-B) of mortality. Longleaf pine sustained the lowest level of seedling mortality (Table 4) and the near level (i.e., zero slope) nature of the longleaf pine mortality curves (Figure 2) illustrates the generally stable survival of this species throughout the test period (subsequent to the weevil infestation). The slight rise in longleaf pine mortality over time was largely attributable to 2nd- and 3rd-year infections by the brown spot needle blight fungus. The rate and level of mortality of CSP seedlings was intermediate between that of OSP and longleaf seedlings (Table 4, Figure 2).

Dead and dying sand pines consistently exhibited root resinosis typical of that displayed by seedlings infected by *P. cinnamomi* (Barnard 1988), and the fungus was consistently isolated from roots of these seedlings (Figure 3). In contrast, root resinosis was generally absent in dead/dying longleaf seedlings, and *P. cinnamomi* was only occasionally isolated from longleaf roots in the laboratory. In such cases, the occurrence of the fungus was considered to be a function of saprophytic colonization of dead roots and/or incidental rhizosphere infestation.

The continuous rise of the sand pine mortality through age 3 (Figure 2), the associated occurrence of root resinosis, and the consistent isolation of *P. cinnamomi* from roots of dead and dying seedlings (Figure 3) indicate a marked susceptibility of sand pine to *P. cinnamomi*. Ross and Marx (1972) reported CSP to be more susceptible than OSP to *P. cinnamomi* under greenhouse conditions. In our study, nearly twice as many OSP apparently succumbed to the pathogen, despite the fact that we recovered more *P. cinnamomi* from the rhizospheres of CSP (Table 2). This relatively poor performance of OSP as compared to CSP under field conditions is in agreement with observations made by others (Burns 1968, 1972, Hebb 1982, Ross 1970). However, our study provides the only direct comparison of the two varieties in the field under measured challenge by a proven root disease pathogen. Previous workers (Balmer 1973, Brendemuehl 1981, 1991, Burns 1968, 1972, Ross 1970) have attributed differential field performance of the two varieties to differences in resistance or susceptibility to mushroom root rot, caused by *Armillaria tabescens* (Scop.) Dennis, Orton & Hora [=*Armillariella tabescens* (Scop.) Singer, =*Clitocybe tabescens* (Scop.) Bres.], despite the fact that the pathogenicity of *A. tabescens* on sand pine has not been established (Ross 1973). Our data, the proven pathogenicity of *P. cinnamomi* on sand pine (Ross and Marx 1972), and a growing body of evidence (Barnard 1988, Barnard et al. 1985 a, b, Ross 1973, Ross and Marx 1972), suggest that *P. cinnamomi*, not *A. tabescens*, is the more important pathogen of sand pine. Results of our study also demonstrate that planting sand pine on deep sandy soils is no

Table 4. Average survival and growth measurements for longleaf and sand pines after 3 yr on a *Phytophthora cinnamomi*-infested deep Lakeland sand in West Florida.[1]

Treatment	Survival (%)	Stem height (cm)	Basal stem diameter (cm)	TVI[2] (cm^3)×(10^3)
Longleaf	64.0ab	41.9bc	3.7a	46b
Longleaf/benomyl	65.6ab	46.8bc	3.9a	58b
Longleaf/Pt	73.6a	32.4c	3.1b	29c
Longleaf/benomyl/Pt	74.4a	57.1b	4.1a	89a
Ocala	16.8c	146.0a	2.9b	26c
Ocala/Pt	18.4c	142.4a	3.0b	29c
Choctawhatchee	50.4b	143,7a	3.2b	93a
Choctawhatchee/Pt	49.6b	135.9a	3.1b	81a

1 Values within columns sharing a common letter do not differ significantly at $P \leq 0.05$ according to ANOVA and Duncan's multiple range test.
2 TVI = Treatment volume index = Average number surviving trees per treatment plot (0.014 ha) x average stem height (cm) × average basal stem diameter^2 (cm^2) per treatment.

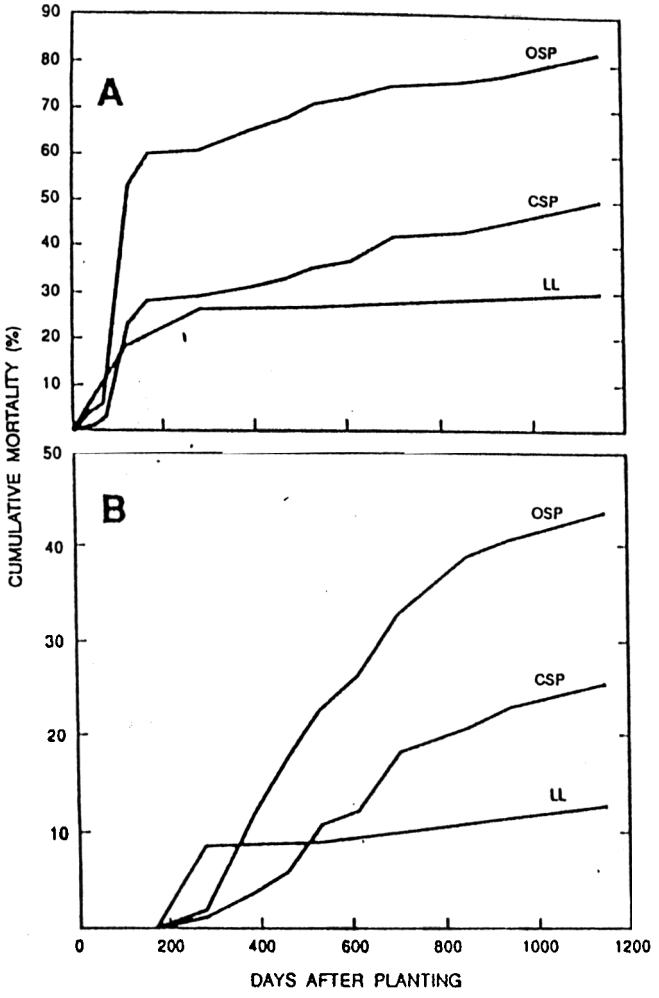

Figure 2. Progressive mortality of Ocala sand pine (OSP), Choctawhatchee sand pine (CSP), and longleaf pine (LL) seedlings on a *Phytophthora cinnamomi*-infested sandhill in west Florida expressed as (A) percentages of the total numbers of planted seedlings, and (B) percentages of seedlings living on sample dates immediately preceding successive site visits and mortality assessments (subsequent to a spring-first-year infestation of pales and pitcheating weevils, coincidental with shaded portion of graphs).

guarantee against *P. cinnamomi*-related losses.

The near level longleaf mortality curves (Figure 2), the absence of root disease symptoms on this species, and infrequent isolation of *P. cinnamomi* from roots of dead or dying trees suggest resistance within longleaf pine to infection by *P. cinnamomi*. Longleaf pine has exhibited relatively high resistance to both fusiform rust (Powers 1975) and annosus root rot (Hodges 1974, Kuhlman 1970). Zak and Campbell (1958), however, were unable in liquid culture to demonstrate meaningful resistance within this species to *P. cinnamomi*, and field resistance of longleaf pine to *P. cinnamomi* has not been documented previously. Clearly, our data suggest that longleaf pine should be preferred above sand pine for plantings on *P. cinnamomi*-infested sandhills.

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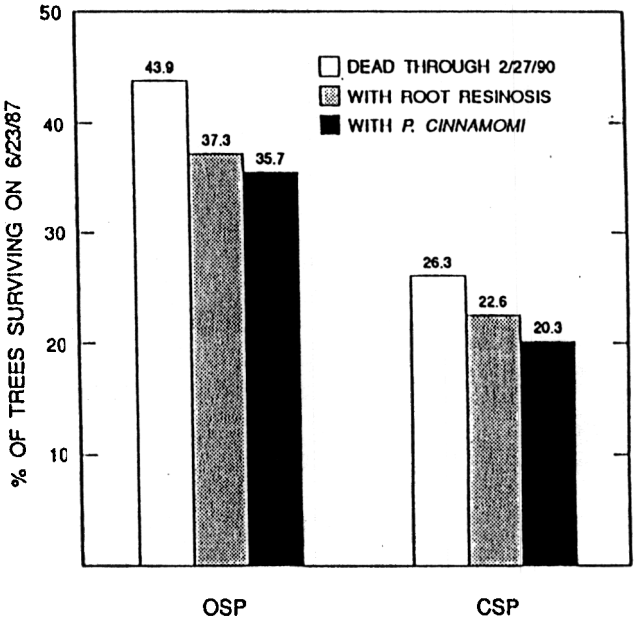

Figure 3. Association of root resinosis and *Phytophthora cinnamomi* with mortality of Ocala (OSP) and Choctawhatchee (CSP) sand pine seedlings on a west Florida sandhill.

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Announcements

Twenty-Second Southern Forest Tree Improvement Conference. Forest Genetics in a Changing World, June 14–17, 1993. James L. McConnell , Chair, SFTIC Committee, 1720 Peachtree Rd. NW, Suite 816 N, Atlanta, GA 30367 (404) 347-4045.

Seminar Series—Field Data Recorders: Hardware and Software. Forest Resources Systems Institute (FORS), 122 Helton Court, Florence, AL 35630 (205) 767-0250; FAX—(205) 767-3768.

NIH Training Program in Molecular and Quantitative Genetics. North Carolina State University announces the availability of National Institutes of Health *Predoctoral Traineeships* to highly qualified individuals for graduate training at the interface of molecular, developmental, and quantitative genetics. The training program focuses on understanding the molecular and developmental basis for variation in quantitative traits. The program is broadly based and includes faculty from five different academic departments. Graduate traineeships will be awarded to research leading to a Ph.D. degree in the Department of Genetics. Applicants must be citizens, noncitizen nationals of the United States, or have been lawfully admitted for permanent residence. These awards carry a stipend of $13, 200 per year plus tuition and a research allowance. Information packets and application forms required for admission to graduate school can be obtained by writing to Ms. Joyce Clayton, Admissions Secretary, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614. (FAX: (919) 515-3355.

New Publications. Five new publications are available, without charge, from Information Center, USDA Forest Service, Suite 850, 1720 Peachtree Road, Atlanta, GA 30367:
1. *The Two-Aged Stand*, Manage. Bull. R8-MB 61.
2. *Stream Improvement Handbook*, Tech. Publ. R8-TP 16
3. *Firewood Calculator*, rev. 1992
4. *Firewood Worth Calculator*, rev. 1992
5. *Fuel Value Calculator*, rev. 1992

Computer Program Release. The National Hardwood Lumber Association has released HaLT (Hardwood Lumber Training), a self-taught training exercise for students, beginning lumber salesman, beginning lumber inspectors, sales and office personnel. *Contact:* Anne Rowland, NHLA. 1 (800) 933-0318.

(Send announcements to Karen Winget, 184 Gold Kettle Dr., Gaithersburg, MD 20878 or FAX (301) 963-0537.)